REMARKS

The Office Action divides the claims into four groups, I-IV and asserts that each group is drawn to a distinct invention.

In particular, with respect to groups I-III, the Office Action asserts that:

> Inventions I, II, and III are directed to related methods. The related inventions are distinct id the inventions as claimed do not overlap in scope, i.e., are mutually exclusive; the inventions as claimed are not obvious variants; and the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect. See MPEP § 806.05(j). In the instant case, each method amplifies a different number of templates, with differing amounts of primers and dyes. Each method measures differing emissions at differing points to differing ends. Each uses differing calculations to arrive at emissions total.

Applicants respectfully traverse the division of claims 1-61 into three groups. Applicants in paragraph 47 of the present application state (*emphasis added*):

> Combining the property of double stranded DNA dyes with the unique melting temperature of each amplicon has led to unexpected advantages of using these inexpensive dyes to conduct multiplex real-time PCR according to methods described in the present invention. When a PCR reaction temperature rises from the annealing and/or extension temperature to a denaturing temperature, the amplicon with the lowest T_m unwinds first, the amplicon with a next higher T_m separates next, and the amplicon with the highest T_m denatures the last. Concurrently, the fluorescent emission of a double stranded DNA dye changes in proportion to the rising reaction temperature due to the incremental melting of the amplicons. The difference between two emissions, one taken at a measuring temperature below the T_m of an amplicon when the amplicon remains in duplex and the other taken at a measuring temperature above the T_m when the double stranded DNA of the amplicon unwinds, reflects the emission amount of the amplicon in duplex. The emission amount can be plotted over the number of cycles and the absolute or relative amount of the starting copy number or amount of the nucleic acid template can be determined by methods known in the art. By the same principle, it will be readily appreciated in the art that the emission amount for each amplicon in the single multiplex PCR reaction can be determined by the difference between the emission taken at a measuring temperature

below a T_m and the emission taken at a measuring temperature above the T_m.

The claimed inventions in claims 1-61 are directed to, *inter alia*, determining cycle-by-cycle the emission difference of each amplicon in a PCR reaction between the emission taken at a measuring temperature below a T_m and the emission taken at a measuring temperature above the T_m. The claimed inventions are not mutually exclusive; as they stem from the same principle. The various numbers of amplicons, various primers, dyes, T_m, and calculations are variants of the claimed methods. Additionally, the claimed inventions do not have materially different design, mode of operation, function, or effect, since the emission difference of each amplicon is determined regardless of whether a PCR reaction contain a single amplicon (claims 1-8), or two amplicons (claims 9-28), or a plurality of amplicons (claims 29-61). For example, in claims 29-61, when n=2, claims 29-61 overlaps with claims 9-28.

In light of the foregoing, Applicants respectfully present that the inventions of claims 1-61 are not independent or distinct and request that the restriction on claims 1-61 (group I-III) be withdrawn.

However, to expedite the prosecution of the present application, Applicants provisionally elect claims 9-28 for examination.

If Applicants can do anything more to expedite this application, Applicants request that the Examiner contact the undersigned at (310) 788-3219.

Respectfully submitted,
Perkins Coie LLP

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James J. Zhu, Ph.D.
Registration No. 52,396

Correspondence Address:
Customer No. 34055
Patent - LA
Perkins Coie LLP
P.O. Box 1208
Seattle, WA 98111-1208
Telephone: (310) 788-9900
Facsimile: (310) 788-3399